A CASE OF POLYMORPLISM IN ASPLANCHNA, SIMULATING MUTATION. II

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I may next state some further observations which I was able to verify again and again in regard to the transition of one form of the species to another. As I have said, forms intermediate between the saccate and the humped, between the humped and the campanulate, and even between the saccate and the campanulate, occur. This statement applies to external body form, and to some extent to the nephridia and other internal organs, with the exception, however, so far as I have yet observed, of the size of the contractile bladder. This latter seems to have its large size only in the small saccate type, and I have observed no indications of gradual transitions to the form possessed by the larger rotifers. More significant, however, is the case of the trophi. I have examined these in a great many individuals that in body form were more or less intermediate between the different types; but in nearly every instance the variations of this organ seem to be abrupt and discontinuous; the trophi are either of one type or the other. The only instances found that in any sense transgress this statement were the trophi of a few saccates produced as the result of the slow degeneration of the larger form, in the culture which I have before mentioned. These animals showed trophi that had plainly lost a number of the more delicate characters which I had otherwise found universal through such a wide range of material, and, simultaneously with this, they had become in a degree transitional between the two types; the angle of the inner tooth and a slight crossing of the tips plainly related them to the cannibalistic type, while in size and general form they

belonged to the other. I will record here also two instances in which I have found the cannibalistic trophi in the humped rotifer. The two specimens were almost the last humped individuals found in a culture on the verge of extinction through cannibalism; they were probably the progeny of campanulates. Both were large specimens of the humped type, one showing a rather heavy corona and rather small humps, and being, therefore, in some sense, of a transitional character. It bore campanulate trophi 257μ long—rather undersized—in which, however, the large lamellate teeth of this type were even unusually developed. The other bore typical campanulate trophi 270μ long, These animals contained large unborn humped rotifers of normal type, with normal trophi of 154μ and 170μ, respectively. In these two instances, therefore, the transition from the campanulate to the smaller type occurred one generation sooner in general body form than it did with the trophi, thus emphasizing the partial separateness and non-correlation between the variations in these differently formed structures.

Next as to duration of transition periods and the number of transitional individuals. If conditions are favorable the periods are very brief and the number of transitional types so few that they are readily overlooked unless careful search is made at just the right time. The entire population of a teeming *Asplanchna* pond readily changes from the saccate to the humped type in one week. As before said, the saccates give birth directly to forms with well-developed humps, and these humped young may be at birth as large or even larger than the parent type. One more generation of growth and reproduction may then give large-sized, fully typical humped individuals. Along with these abrupt transitions there usually occur, however, a lesser number that are a little more gradual. Individuals occur like saccates in all respects save that they possess the inconspicuous dorsal hump; others are small with the lateral and ventral (posterior) humps

scarcely showing at birth, but developing to a moderate extent rapidly afterward.

With less favorable conditions the transition is prolonged and the number of intermediate individuals is greatly increased. I have observed no instance, however, in which the species remained for longer than two weeks in a chaotic condition. Either the transition is soon effected or the numbers rapidly decrease and the species disappears.

Nearly similar statements may be made with regard to the transition from the humped to the campanulate type. As already stated, its advent usually occurs by the appearance of a very few individuals with the utmost abruptness. Aside from the fact that the ontogeny is here somewhat more extended—they, the young, being considerably smaller than the adults, with much less expanded corona—there is apparently little, if any, sense of transition. I think it probable that the humped individuals which actually give rise to the very first campanulates are individuals of somewhat extra size and vigor. Such individuals have been found at different times as well as in the case of the two mentioned in my first investigation, but their actual production of the young cannibals has not been observed. In any case their deviation from the ordinary humped type is not great and the usual transition has all the abruptness that the most pronounced mutationist could anticipate. Moreover, as long as the species is thriving and reproduction copious, the two forms remain separated from each other as sharply as do the most distinct species. This is the most frequent condition by far in which one finds them.

When, however, conditions become less favorable, which fact usually means that the food supply of the humped form is failing, a change intervenes. The humped individuals usually remain quite as they were, without reduction in size or loss of other characteristics, save a much slower rate of reproduction. But this reduces numbers, and especially the number of young.

The cannibals can and do ingest their full-sized congeners, but they are by no means successful in every attack. One may observe them, with empty stomachs, making scores of furious but futile attempts at capturing their adult neighbors. It is largely the young hump-bearers which, though nearly full grown, fall ready victims to the all-embracing coronæ of the cannibals. Thus it follows that any reduction of the food supply of the lesser type immediately impoverishes the larger one as well. The consequences of this are curiously dissimilar in different cases, although always one of two results intervenes. The cannibals may become even more cannibalistic, destroying the entire humped population of all ages, and their own young as well, until the culture is finally obliterated by the death, from old age, of a few veterans which are without further food supply. This has happened again and again in my large culture dishes.[7] In a few cases it has happened that a culture, when at the point of extinction, would again revive by the multiplication of the humped form. This is due to the fact that the last starving cannibals, reproducing, as they always do, both their own type and the humped type as well, fail to eat up perhaps a single member of their humped progeny, which then survives to start a new cycle under less strenuous surroundings. I have carefully followed this decline and survival as thus stated.

In about half of the cases, however, a very different effect is registered upon the campanulate form by the lessening food supply and the falling numbers of the other type. It undergoes a considerable degeneration, which may perhaps reduce it to the form from which it arose, although I have not been able to fully demonstrate this. But forms more or less intermediate are produced by the starving cannibals. The trophi remain typical, but the enormous coronas, as well as the breadth of the entire animal, are much reduced. In the single instance already mentioned in which degeneration gradually reduced the humped type to a small size, and finally to the

[7] I have recently observed one very similar instance in nature.

most diminutive saccate form, I was surprised to find that the campanulates present in the culture degenerated, pari passu, with the other type. They became much smaller, lost their flaring coronas, and nearly every sign of their outward specialization. Yet, generation after generation, they maintained their cannibalistic habits, their heavy musculature, and above all the campanulate type of trophi, the only change in these latter organs being a reduction in size.

In the main, then, transitional periods are brief; transitional forms few. Unfavorable conditions prolong somewhat the existence of both. But the species always is soon eliminated or sets up a new equilibrium under the new conditions.

A few words further may be added at this point upon the matter of fluctuating variation shown by the different forms of this species. Without recording such variations mathematically, I have endeavored to ascertain as fully as possible the answers to three questions: First, how great is the amount of such variation? Second, is fluctuating variation especially correlated with one or other of the types of heterogenesis above described? And third, what causes are operative in producing it?

As to the amount of fluctuating variation, certain facts have already been mentioned that come under this heading. I will but add here the general statement that each of the three types is, in itself, highly variable—quite sufficiently so to be regarded as a decidedly variable species were it really an independent form.

As to the second point, the question of the correlation between fluctuating variation and the mutation-like transitions, this has also been partially discussed under the heading of transitional types. But it is necessary to add the unqualified statement that no evidence has been discovered for such correlation. Variation is one thing; heterogenesis another. The two phenomena contrast, rather than are related. Thus the transition from the saccate to the humped rotifer is often made when the

saccate type is in its most typical condition, at least so far as form is concerned. Of all the minor fluctuating forms found among the saccates the one that most suggests the humped type is that which I have characterized above as urn-shaped. The bulging sides of such a form might readily be thought to be the forerunners of at least the lateral humps; but during the entire study I have been unable to observe the humped form originating from this urn-like variety.

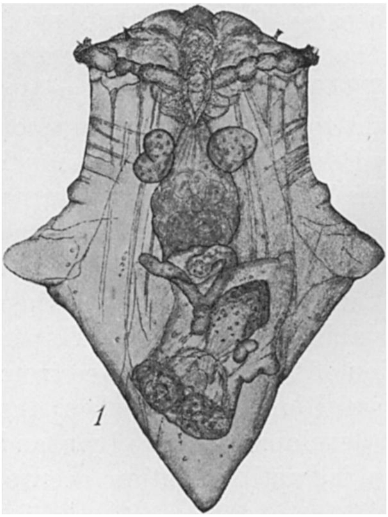
Furthermore, the saltations from type to type do not necessarily occur, and I think do not usually occur, when the amount of fluctuating variability is greatest. This is especially true of the formation of the campanulates from the hump-bearers. This transition occurs when the latter are at a culmination of development and vigor, and in this condition the species is, until saltation occurs, relatively uniform.

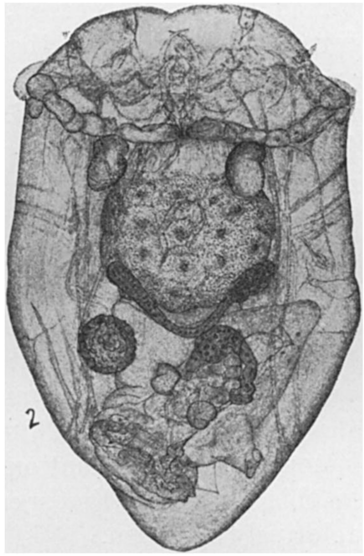
Under the third question, as to causes of fluctuating variability, I will record at present but two points, one general and one special.

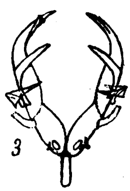
The greatest amount of general fluctuation seems always to occur under relatively unfavorable conditions. Favorable conditions, on the other hand, tend to produce full development with relative uniformity among the individuals of any given type.

One special instance of variation interested me so much that I followed it whenever found, in the effort to get at its exact cause. This is the variation in the length of the three conspicuous humps which characterize the commoner form. The amount of this variation is, although I have not measured it, very great. The humps may be but angular projections upon the body's outline, or they may elongate until they might be appropriately described as finger-shaped. The type represented in the illustration, Fig. 1, may be taken as typical. This type is repeated in countless numbers, with but moderate variation, so long as conditions are normal, which means chiefly, so long as the food supply is uniform and ade-

FIG. 1.







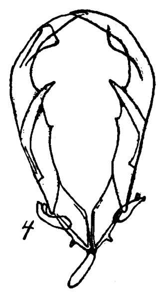


FIG. 1. *Asplanchna amphora*, OF THE HUMPED FORM (FORM *B*), SHOWING TYPICAL YOUNG DEVELOPING PARTHENOGENETICALLY WITHIN THE BODY OF THE PARENT. Magnification about 8 diameters.

FIG. 2. *Asplanchna amphora*, campanulate form (form *C*), showing heterotypic young developing parthenogenetically within the body of the parent. This figure is drawn from a specimen chosen because it exhibited well the heterotypic reproduction. In other respects it does not represent the true form of the campanulate type, the shrinking action of the killing fluid having both shortened and contracted the corona, obliterating thus the bell-like form and rendering the anterior portion highly convex instead of concave as in life. The convex end of Fig. 1 is, on the contrary, quite correct, the smaller type being much more easily killed in perfect form. Magnification about 8 diameters.

FIGS. 3 AND 4. Trophi of humped and campanulate forms, respectively. The contrast in size is inadequately shown by these figures. Had average specimens been chosen, Fig. 4 would have been twice the length of Fig. 3. In both figures the "accessory jaws" are omitted, they being so weakly developed as to be quite destroyed in the process of extraction. In Fig. 4 the upper lateral pieces, the outriggers, are omitted for the same reason. Magnification about 210 diameters.

quate. The individuals with smaller humps are always transitional or degenerative in origin.

But what could be the cause of the hypertrophy of the humps to fully double their usual prominence, and this in individuals that always gave evidence of starvation? Such individuals occurred in certain cultures in considerable numbers, and constituted a very extreme type; the animals were always much more transparent than any others, the body wall being thin and the internal organs usually pale, shrunken, and undeveloped, the stomach empty, and embryos lacking. The general body form was extremely slender, with corona but two thirds average width, while the ventral, or rather posterior, hump was not only long, but developed a secondary prolongation, as it were, from the end of the original one. Such animals swim, all but habitually, with the lateral humps retracted, and in this condition are so slender as hardly to suggest the genus *Asplanchna,* the form being apparently more nearly that of *Hydatina.* But with the thrusting out of the lateral humps a singular transformation occurs; these structures are so long that their expanse equals or exceeds the animal's length, and so slender that the animal's forward motion bends them backward. In my notes I designated these extreme animals as the "cross-bow type."

As already mentioned, they occurred in considerable numbers in several of my mass cultures. I also obtained them several times under controlled conditions in isolation experiments, but I found no clew to the cause of their production until I discovered that they were frequently produced by the gaint campanulates, and especially by the campanulates that were *Moina*-feeders. It seemed very striking that this variant, which carried the development of the humped form to its utmost extreme, should be thus produced by the robust companulates in which there are no humps, and in which, indeed, all the characteristics are at the farthest possible remove from the type in question. Yet these cross-bow hump-bearers

formed a regular part of the progeny of the massive crustacean-feeders. All but invisible, they swam restlessly about seeking for available food, which was not present, until many of them fell victims to the greedy members of the parental stock.

This combination of overfed parent and foodless progeny offered the suggestion of the cause I was seeking, which was then readily confirmed by experiment. Maximum nutritive conditions before birth and the entire absence of available food for at least 24 hours after birth produces the slender transparent type with the hypertrophied humps. Under these conditions the body wall, and its projections, which are highly developed even at birth, continue to develop for a considerable time afterwards, undoubtedly withdrawing nutrition from the internal organs—stomach, digestive glands, ovary, etc.—these thereby undergoing a partial atrophy.

A certain interest attaches to this explanation, because it not only furnishes the rationale of an extreme type of fluctuating variation in this rotifer, but because the facts closely parallel the incidents in the development of the male of the same species. The males at birth lack, of course, the chief internal organs of the female, and can not draw upon them as sources of nutrition, but they do draw upon the rudimentary digestive tract until, before death, it has frequently quite disappeared. Moreover, the males undergo a progressive development of the body wall and to some extent of the humps during the two to four days of their active life. The male thus becomes more differentiated in the active portion of its organization, absorbing meanwhile what little inactive tissue there is to absorb. The same thing happens to the young foodless female, save that there is more tissue to absorb, and the process is not carried so far.

Sufficient investigation would doubtless unravel each of the other minor fluctuations which the three forms of the species undergo, and most of them will all but certainly resolve themselves into factors of nutrition. Few

species of animals are capable of so numerous and varied nutritive transitions and respond to them in so fundamental and varied manners as does this *Asplanchna*.

Before closing this paper it becomes a disagreeable necessity to attempt some more definite systematic placement of the forms of *Asplanchna* here discussed. The task is a difficult one for several reasons. In the first place, it is obvious that the facts here recorded tend to disturb our very conception of what constitutes a species in this genus. If we accept the interpretation of polymorphism as, on the whole, a little more applicable to the facts of heterogenesis here cited than would be the interpretation of mutation, the question is obviously raised whether several of the other *Asplanchna* types hitherto described as distinct species may not likewise be closely related genetic forms, connected, as are those here described, either with each other or possibly with these very types. Thus a relationship is readily thinkable between *A. ebbesbornii* and *A. intermedia* or *A. sieboldi*, or possibly of one or the other with *A. brightwelli;* although the disparity of the males in this last type renders relationship less probable.

Moreover, in the literature of the subject the claim has been made at least once that such a relationship exists between European forms, as the following quotations from Wesenberg-Lund[8] in which he cites Daday to this effect, will show:

> Uber die Fortpflanzungsverhältnisse der Asplanchnen kann ich Folgendes mittheilen.—v. Daday ("Ein Fall von Heterogenesis bei den Räderthieren." *Mathem. und Naturw. Berichte aus Ungarn,* 7. Bd., 1888–1889, p. 140) hat für *Asplanchna sieboldi* einige ganz merkwürdige und bisher exceptionelle Fortpflanzungsverhältnisse geschildert. Seiner Meinung nach findet man hier zwei verschieden geformte Weibchen, theils solche, die den gewöhnlichen schlauchförmigen *Asplanchna*-Typus haben, theils solche, die den eigenthümlich geformten Männchen dieser Art gleichen; diese sind durch 4 conische Erhöhungen characterisiert, und sind diese Erhöhungen derartig vertheilt, "dass je eine auf die Mittellinie des Bauches und der Rückseite, eine auf die rechte und eine

[8] Wesenberg-Lund, C., "Ueber danische Rotiferen und über die Fortpflanzungsverhältnisse der Rotiferen," *Zool. Anz.*, 1898, Bd. 21, pp. 200–211.

auf die linke Seite fällt, wodurch, von vorn betrachtet, die Form eines gleichschenkeligen Kreuzes sichtbar wird" (Daday, p. 153). Jedes dieser zwei verschieden gebauten Weibchen vermag sowohl Weibchen ihrer eigenen Gestalt parthenogenetisch hervorzubringen, als auch Weibchen der anderen Art; ferner auch Männchen und, nach der mit diesen erfolgten Begattung Dauereier (pp. 206–207).

Daday's work on the rotifers has in general been frequently criticized and all but discredited, and this observation on his part of a reciprocal relationship between *A. sieboldi* and a saccate *Asplanchna* fares no better at the hand of Wesenberg-Lund. He replies that he has himself reared *A. sieboldi* in an aquarium for a month, that he has studied them thoroughly and has found no such reproductive phenomena. He continues, that Daday has simply been mistaken in his observations, having failed to distinguish the humped *A. sieboldi* from the saccate rotifer, because the humps of the former species are often retracted, giving it for the moment a saccate form.

This criticism of Daday's reported observations may of course be correct, but it seems as naïve as it is severe. It is of course true that the humped rotifers retract the lateral humps; but the position of these protuberances always remains marked by folds of the body wall, while the ventral hump is not retracted at all. Daday must indeed have been a poor observer to be thus deceived, the more so, in that the moment these animals are placed under the pressure of a cover glass or even in a very shallow drop of water on a slide, the pressure of the cover glass or their own weight forces them to expand the humps and instantly reveal their type of structure. In the light of my own study it seems far more probable that Daday was correct in his reported observations than that Wesenberg-Lund is correct in his criticism of them. The fact that Wesenberg-Lund reared the humped *Asplanchna* for a month without the occurrence of heterogenesis is of no especial significance. The writer has reared the humped form discussed in the present paper for longer periods than this with the same result.

Heterogenesis is confined to special periods or caused by special conditions as above set forth.

However, not only is the question of species confused by the presence of heterogenesis in the genus, but another difficulty which I had not anticipated manifests itself; viz., the discovery that the descriptive work which has already been done upon the genus has not, even the best of it, been sufficiently accurate to be trustworthy. I make this statement with the utmost reluctance, and only after I have spent weeks of effort to bring my observations upon single points into accord with the statements of Rousselet, who is not only the highest authority on the group in question, but who has, as already stated, made the last and most detailed pronouncement upon the species of the genus. I have failed, however, in my efforts. Nor are the discrepancies such as may, with probability, be explained by the assumption of differences in the material which we have examined. Thus, to take an example from Rousselet's description of the jaws. He says:

> At the tip there is really but a single point . . . ; on crushing the jaws a thin, chitinous ridge seen in side view is bent over and simulates a second tooth.

Hudson, in his supplement, has also spoken to similar effect, viz.:

> When the ramus is subjected to pressure from above the deep plate is bent by the glass (to which it stands at right angles), and its free lower corner is twisted, so as to look sometimes like a second tooth, just below the extreme apex, sometimes like a small plate.

Now in spite of these statements I seemed to see the thin lamellate plate-like second tooth near the apex of every jaw in case the conditions for its vision were at all adequate, and in the giant campanulate this structure, so delicate in the ordinary type, becomes greatly developed, forming a large cutting tooth, which, when the jaws are closed, meets with its fellow of the opposite side, in the middle line. That this thin triangular tooth in the ordinary form was a false appearance produced by the

bending over of the corner of a ridge by the pressure of a cover glass seemed improbable, and I immediately put it to test in various ways. For one, I extracted a large number of the trophi by means of potassic hydrate in deep watch-glasses. Here there was obviously no pressure, yet the structures in question were quite visible, even before the trophi had been transferred to a slide, or touched by any instrument. Moreover, these thin lamellate teeth are never quite symmetrical on the two rami, and this delicate discrepancy is always on the same side of the animal, as I ascertained later in stained and mounted preparations. I carried my study of the trophi farther by mounting many which I had extracted in deep hollow-ground slides, including with them a small bubble of air. By giving the slide a quick tilt the air bubble could be made to strike and overturn the trophi in different ways. By then replacing the slide quickly under the microscope, views could be had of the trophi before they had settled to the ordinary horizontal position. A half hour of such attempts readily furnished views of every part of the trophi, seen from almost every possible angle. Portions so thin as to be invisible in one view become visible in another; optical sections at all points make possible the arrival at the correct form. I regret that in my drawing I have been able to show so little of the delicate complexity of these structures; but Rousselet's view of their structure—that "the chitinous material is bent at right angles throughout the length of the rami, forming an inverted L in cross section"—is certainly very far from correct. The structure varies at different points; ridges thicken and fade out in complex and sinuous fashion, quite as we should find them in the complex chitinous jaw of an insect, or, for that matter, in the jaw-bone of a mammal.

The tips of the jaws are interesting, and I find no description of the jaws of any species of *Asplanchna*, by any author, which coincides with my observations. Nevertheless, this may be due to the inadequate study of

these difficult structures by systematists who can devote but little time to a given point. I find the two rami are never alike at the very tips. I am not speaking now of the delicate lamellate teeth already mentioned which are a little distance removed from the tips, but of the very extremities. Of these latter, one is bifid, or ends in two delicate tips; even these again are never quite symmetrical, but the one which is toward the animal or posterior is a little smaller and shorter. Furthermore, the split in the tip of this jaw is not a simple cleft such as one might produce by splitting the end of a stick with a knife, but is a triangular groove, the base or open side of which is toward the inside or concave aspect of the ramus, the apex toward the outside. As aforesaid, this cleft divides the tip of the ramus, but it is also continued on the inner aspect of it considerably farther than it extends on the outer, becoming thus shallower and shallower as it extends farther from the divided tip. The opposite ramus is not bifid, but tapers to a point, and the tapering is of such a nature that the jaw near the tip is more or less triangular in cross section, so as to fit, not only into the cleft between the tips of its fellow ramus, but farther into the triangular groove on its inner side as well. Thus these delicate chitinous jaws, when closed, lock together in double manner.

The study of hundreds of examples of the trophi of the humped rotifer as it occurred in the material first examined left upon the mind of the writer a very distinct impression of the minute delicacy of detail and very great uniformity which prevails in these structures. Variability seemed almost wholly confined to the matter of size.

Turning briefly to the trophi of the campanulate type, I will say that they differ regularly from those just described, not only in the features shown in the figure, such as general size, breadth of rami, more acute angle of the lower inner tooth, etc., but in other marked features besides. The inner tooth, smaller in proportion as well

as set at a different angle from the corresponding structure in the humped type, is here fused with the ramus instead of being merely bent over inward from its outer margin. But the tips differ most; the secondary lamellate teeth, as before mentioned, become very large, though variable, structures. They always meet in the middle line when the jaws are closed; they have wavy or corrugated surfaces, and thin down to a sharp cutting edge. The tips of the rami are modified most of all. They are slender and greatly extended in length, meeting and passing at an acute angle. Neither tip is bifid, and the asymmetry between the two rami is much less marked. The jaws do not interlock, when closed, in the sense in which they do in the humped type; instead, the tips invariably cross, like the mandibles of a crossbill, the farther closing being prevented by the meeting in the middle of the lamellate teeth. Occasionally I have noticed a campanulate whose jaws had sheared past in the wrong way; the lamellate teeth then did not meet to prevent farther closing, and the animal had apparently lost control of the organs, as the two halves remained crossed well down to near their bases.

Returning to Rousselet's description of the trophi of *A. amphora,* which must, of course, be compared only to the trophi of the humped type, I will say that despite the discrepancies in detail which I deem due to inaccuracy of observation, it remains true, nevertheless, that his general figure of the trophi of this species coincides essentially with the general appearance of the trophi as I find them in the humped and saccate types, and this constitutes a fair reason for assigning, provisionally, the material which I have studied to the species *Asplanchna amphora.*

However, I can not leave this matter of the trophi without instancing a surprising observation which I have made the past summer on the trophi of the related species, *Asplanchna brightwelli*—an observation which again complicates, in an entirely new way, the question of species in the genus *Asplanchna.*

As soon as I had discovered the fact that the humped *Asplanchna* which I was studying was represented by a saccate type which in many characters approached closely to *Asplanchna brightwelli,* I began an extended search for this latter species, in order to study closely the question of relationship or non-relationship. At different times, throughout a period of one year, I succeeded in finding *A. brightwelli* in five different ponds in my own vicinity. The species tenants ponds of a different character from those in which the larger *Asplanchna* flourishes, and is associated with a somewhat different micro-fauna. In but one instance have I found the two species developing together, and in this case the larger *Asplanchna* did not pass beyond the saccate condition, in which it was also present but sparingly. The resemblance of its occasional representatives to the more numerous and likewise saccate individuals of *A. brightwelli* was so great as to almost prevent its detection. Only my constant work with the larger species could have sharpened my attention to the point of noticing any especial lack of homogeneity in the material. Yet the saccates of the larger species were regularly a little longer and about one fourth broader than the adult *A. brightwelli.* They differed also in a number of minor constant characters. But none of these have hitherto found place in any specific descriptions, with the exception of the difference in the trophi. The trophi of the larger saccates agreed with the description and figure which I have given in everything save that they were a little undersized. The trophi of *A. brightwelli* agreed in their general outline with the figure given for this species by Rousselet; they possessed the more delicate, perfectly oval contour, and invariably lacked the large inner tooth, just as Rousselet asserts that he has always found them to do. Into the question of the form of their tips and the presence or absence of the all but invisible lamellate teeth I do not go. Such study as I have given them led me to think that they were constructed in these respects

essentially, but not exactly, as were those of the type which I had already studied. I was much pleased to thus substantiate, at least in a general way, on this American material, Rousselet's judgment on the distinction between the trophi of these two species. I will add that a score of culture experiments started with single individuals of the two types fully confirmed their distinctness. Despite their very close resemblance, I reared from one set of the delicate saccates the humped *amphora* type with which I was so familiar; while parallel cultures, with identical conditions as to food and temperature, produced no modification in the *A. brightwelli* other than a slight increase in size.

I therefore reached the conclusion that, delicate as are the differences which separate the saccate form of *A. amphora* from the invariably saccate *A. brightwelli,* they were none the less sharply demarcated. Ignoring other features, it seemed perfectly safe to trust the one character of the absence or presence of the larger inner tooth on the trophi.

Imagine my surprise, then, when, upon visiting an entirely different locality—Custer County, South Dakota—I discovered an *Asplanchna* in countless numbers which completely upset this distinction and introduced me to a seemingly new type of variation within the genus.

It was in the charming little mountain lake (or rather reservoir, for the original site contained a mere pool which has now been increased to a depth of 80 feet by an artificial dam) called Sylvan Lake, that I came upon the rotifer in question. The lake was indeed swarming with rotifers of different species, which constituted the majority of its plankton. Monarch of them all, and profiting greatly by its superior size and ingesting power, was a superb *Asplanchna.* Aside from a slight excess in size every outward character indicated *A. brightwelli.* Moreover, I had found the species in the very nick of time, for both males and resting eggs were copiously present. Among very large numbers of these which I

immediately examined not one differed outwardly from the *brightwelli* type. But examination of the trophi yielded the astonishing result that in every instance they bore a strong inner tooth in the exact position in which this is found in *A. amphora.* I examined large numbers of them and found that in all the features which I could study with the facilities I had in the field, there was no obvious variation whatever. It should be mentioned that in outline these trophi presented the close approach to a perfect oval which is characteristic of the *brightwelli* type. The strong inner tooth alone gave them decidedly the aspect of the jaws of *Asplanchna amphora.*

This discovery is plainly again confusing, as to specific distinctions between the types. Fortunately, however, it serves at least to clear up certain contradictions in the literature of the subject. Rousselet, in the article above mentioned, dealt especially with this point. He figures the jaws of *A. brightwelli,* to use his own expression, as he has "invariably found them"—*i. e.,* with an oval outline and without the inner teeth. He concludes that the earlier writers on the genus—Dalrymple, Brightwell, Hudson—had certainly confused two different species of *Asplanchna,* describing the trophi of *A. amphora* as belonging to *A. brightwelli.*

My examination of the Sylvan Lake material shows that no such error need be ascribed to them. *A. brightwelli* simply exists in two distinct races (genotypes?); Rousselet has invariably found but one of these, just as I myself have done in my own vicinity; while Brightwell very probably found and described the other, which I have found so abundant in the South Dakota lake.

The finding of *A. brightwelli* with two distinct types of trophi may seem but a trivial matter, but taken in its full connection it is not without interest. *A. brightwelli* seems, in general, an all but constant species. Yet, judging by morphological test, it should be closely related to *A. amphora,* a species which experiment shows to be phenomenally variable.

What, then, is the relationship—the physiological and genetic relationship—between these two types? Jennings in his recent work on the "Characteristics of the Diverse Races of *Paramecium*" has prophesied that the more exact study of the life history of rotifers will demonstrate that much of their apparent variability is really due to the presence, within specific limits, of numerous fixed races.

Now the study, as here outlined, of the variation of *A. amphora*, brings to light a condition which in no wise substantiates this prophecy. No fixed races are present; but strongly demarcated yet temporary types, on the one hand, and fluctuating variations, on the other, which are all or nearly all the result of nutritive stimuli. Is it possible that, in spite of this, the closely related *A. brightwelli* will present the fixed races which Jennings suggests?

I have aleady indicated that a series of about twenty culture experiments with the type of *A. brightwelli* first found by me yielded no significant modification. At the present writing I am again following this species in copious natural development and again conducting a few mass cultures without finding anything but farther proofs of constancy.

I am also succeeding in rearing very large numbers of *A. brightwelli* of the type whose trophi present the inner tooth. The resting eggs, which were brought from Sylvan Lake the preceding August, were kept over winter in a small amount of the lake water and hatched out in March by adding tap water and raising the temperature by placing the dish in the sunlight. The culture medium has slowly been quite changed to the somewhat alkaline and saline water of the writer's locality. The cultures have also been heavily fed upon organisms to which they are certainly unaccustomed in nature. Some cannibalism has been induced. But the trophi remain obstinately true to their own type, and the general morphological changes have been confined to a considerable increase in

size of a few individuals, with perhaps a somewhat disproportionate expansion in breadth of corona. Yet the results of a preliminary six weeks' culture of this type of *A. brightwelli* are essentially similar to those which followed my attempts to modify the first type: they are negative.

Much more extended and varied experiments must be made before reaching final conclusions upon the constancy of these two races of *A. brightwelli.* Yet they certainly promise to bear out in the main Jennings's prediction of relatively fixed races within the species.

Yet the foregoing does not entirely complete the picture of variation as I have found it in *A. brightwelli.* While the rearing of thousands of individuals and the examination of a very large amount of material in nature give the appearance of two stable genetic types, yet in the rarest instances mutation-like changes of the most marked character probably do occur, just as they do in *A. amphora.*

While at Sylvan Lake it occurred to me that the very favorable conditions under which *A. brightwelli* was there developing, including the preying upon a number of different organisms, were as well adapted as possible to bring about mutational changes such as I had found to occur in *A. amphora.* Day after day I examined large amounts of material with wholly negative results. But a favorable morning at the very close of my stay at the lake enabled me to collect several liters of plankton as thick as cream in consistency, with perhaps four fifths of its bulk living *Asplanchna.* Pouring this in the thinnest possible layers, into broad dishes, and placing these above a black surface, I proceeded, by means of a powerful reading lens, to search for any individual *Asplanchna* showing marked deviation from type. Minor deviation could not, of course, be thus detected. To my great surprise, my search was finally rewarded by the finding of three individual rotifers, and three only, of quite astonishing proportions. They were certainly *Asplanchna;*

that they were derived from the *brightwelli* I of course have no proof, but in the light of my study of *A. amphora* it seems probable. They were campanulate forms, differing from the slender saccate *A. brightwelli* even more than the campanulate *A. amphora* differs from the smaller types of its species. Seen in dorsal view, when freely swimming in a drop of water without cover glass, they presented almost the form of an equilateral triangle with one rounded corner; this was the posterior end; the entire opposite side being taken up by the loose flapping corona. I regret that, in my haste, I was unable to study these forms precisely, and much less to prove their relationship. But I hope that the isolated observation may perhaps induce others to seek among crowded stocks of *Asplanchna* of different species for rare and much modified forms. If, as I believe will be the case, they are found to occur occasionally in *A. brightwelli* and perhaps other species, it will throw an added light upon the changes which so readily take place in *A. amphora*. The rarity of their occurrence will render clearer the relationship of the phenomena to the recognized instances of mutation.

Before closing the discussion of facts relative to the specific determination, statements must be made with regard to the males and to the resting eggs. Similar males are produced by all three of the forms which the amphora-like *Asplanchna* assumes. The humped and campanulate types produce them copiously; the saccate type but rarely and at periods when it is about to pass over into the humped form. These males are always of the well-known type bearing two lateral humps. They quite agree with Rousselet's determination, except that he evidently assumes the size to be uniform, whereas I find it to be extremely variable, the limits being as three to one. The largest males, often present in abundance, reach fully the size of the humped females; *i. e.*, a length of 1500μ. The cause for the wide divergence in size is the varying degree of development at birth. This affects them as it affects the young females, except that the young males,

being unable to feed and thus continue their development, are obliged to remain at approximately the same divergent sizes at which they are born.

In regard to the resting eggs, they are, of course, as are the males, produced by all three types, and but rarely by the smaller saccate form. The number produced by one individual varies greatly with the degree of nutrition. But one to three are matured if the females are poorly fed after fertilization; whereas as many as six are frequently present at one time in the body when nutrition is high, and very rarely as many as nine may be seen. The large campanulates usually show a high number, but it does not exceed the maximum produced by the humped form. The color of the egg, which Rousselet uses as a specific character, is variable in this species. In individuals fed on *Paramecia* the eggs are quite white; in individuals reared on *Brachionus* they are light yellow to orange; while in *Moina*-feeders they are dirty white to brown. Again, the volume of yolk, *i. e.,* the filling or not filling of the egg cavity, which Rousselet also regards as important, I find to be highly variable in the eggs of both this species and of *A. brightwelli.* It is partially a matter of the age of the egg; but eggs are frequently deposited in the most different conditions with regard to this character. There remains the size of the egg and the appearance of the egg coats, both of which are highly characteristic and relatively uniform. The size of the egg is much less, relative to the size of the animal, than is the case in *A. brightwelli;* but the actual size is larger and exceeds the dimensions given by Rousselet for *A. amphora,* viz., from about 200μ to 225μ, as contrasted with his figure, 170μ. This is surprising, in that, in the case of *A. brightwelli,* my measurements of the resting egg—170μ to 190μ—is less than the figure—205μ —given by Rousselet.

The egg envelopes, which I have studied in an almost indefinite amount of material, grown under very diverse conditions, are the most uniform and at the same time the

most peculiar feature which I have found in the species. They plainly do not agree with Rousselet's characterization of the egg of *A. amphora:* "The outer shell envelope consists of numerous much smaller globular transparent cells" (smaller than the cells in egg coat of *A. brightwelli*) "through which a finely dotted inner membrane can be seen." I find that at a certain intermediate stage of development a dotted inner membrane can be seen, the dots being the ends of either tubes or rods making up a thick inner coat; the rapidly developing outer shell, however, soon obscures these dots and the coat assumes at first a wrinkled, then a heavily corrugated, surface. The corrugations are so disposed that many of them converge at two opposite poles of the egg. I deem it quite impossible that this characteristic and beautiful structure should have been overlooked by any one studying this species in detail and with the full characters which it possesses in the writer's vicinity. It therefore seems very probable that the type of *A. amphora* studied by Rousselet was not identical with that studied by the writer, and it may accordingly prove necessary to eventually separate the form I have studied from the original type of the species, ascribing it varietal rank, based on at least this one character of the egg coats. The systematic predicament in which this would place these beautiful rotifers would indeed be pathetic or intolerable or humorous, according to our attitude toward things systematic. We should have two varieties, separated from each other by a single fixed character only, and one of these varieties would comprise within itself, besides a host of minor variations, three distinct types, each of which differs from its fellow, not only more than do the varieties differ from each other, but more than the whole species, at its nearest point of approach, differs from its closest congeners.

There is not the least known reason why actual facts of genetic relationship should not be as complicated as this, and if they are so we must deal with them systemat-

ically in some fashion. It is evident, however, that the species in question and other allied forms should be more intensively studied by workers in other localities before we venture upon the final solution of so intricate a question. For the present all that needs be said is that the material studied by the writer and designated by the phrases, the *saccate,* the *humped,* and the *campanulate forms,* belongs to the species *Asplanchna amphora,* as at present constituted; and it seems no less certain that this material is sharply segregated from *A. brightwelli,* despite the exceeding closeness of this latter species to the above mentioned saccate form of *A. amphora.*

A brief résumé of the chief characters of *Asplanchna amphora,* as here studied, will be of use to rapid workers. It is as follows:

Species trimorphic, each of the three forms showing fluctuating variation and occasionally intergradations.

Form *A,* saccate type, produced from resting egg and multiplying by rapid parthenogenesis, through several generations; corona about equaling diameter of body or less, nearly circular in outline, agreeing with the cylindrical body, which rests on side when water is withdrawn; body without humps or with small dorsal hump only; flame cells varying in number from approximately 20 to 40; contractile vesicle large; trophi as in next form, except smaller—about 95 μ to 135 μ long. Length of entire animal 500 μ to 1,200 μ.

Form *B,* humped type, characteristically known as *Asplanchna amphora,* originating from form *A* by rapid saltation and reproducing chiefly its own type; body conical, strongly flattened dorso-ventrally, with one posterior, one dorsal, and two lateral humps of varying size and habit of carriage; corona oval, agreeing with the flattened body, which causes animal to rest on dorsal or ventral surface when water is withdrawn; flame cells 40 to nearly 60; contractile vesicle small; trophi strong, typically from 150 μ to 170 μ in length, though varying from 130 μ to 200 μ, enclosing when closed an area which is not oval but widest in its distal third, with prominent tooth projecting inward seemingly from the inner though really folded over from the outer margin of each ramus, delicate lamellate teeth near the tips and the two rami interlocking when closed by means of one bifid and one pointed tip; accessory jaws, as also in forms *A* and *C,* very weakly developed. Length of entire animal approximately 1,000 μ to 1,800 μ.

Form *C,* campanulate type, originating usually from form *B* as a result of cannibalism, and reproducing both its own form and form *B*;

body very broadly saccate to broadly campanulate in form, with very heavy walls and musculature, strongly flattened dorso-ventrally, never with humps; corona oval and very broad, its breadth frequently equaling the length of the animal; anterior end of animal, within corona, concave instead of convex; flame cells approximately 80 μ to 115 μ; contractile vesicle small. Animal resting when water is withdrawn on dorsal or ventral surface; trophi very large, typically from 300 μ to 340 μ in length, enclosing a narrowly oval area; inner teeth relatively less prominent than in preceding types, set at an acute angle with the ramus and more firmly fused with it than in the preceding types; lamellate teeth near tips of rami much developed and meeting, with cutting edges, in middle line; tips of rami not interlocking but shearing past each other when closed. Length of entire animal approximately 1,800 μ to 2,500 μ.

In conclusion, it may be pointed out that the type of variation shown by the rotifer here discussed seems somewhat peculiar, in that it lies seemingly on the line between germinal variation and variation which is commonly supposed to be somatic. To use recent phraseology, it is difficult to say whether the types which this species of *Asplanchna* produces should be called genotypes or phenotypes.[10] They are like genotypes in that when once produced they manifest a marked tendency toward stability, each type reproducing itself through a number or even a multitude of generations after the special conditions which favored their origin have ceased to be present. They are to some extent like phenotypes in that this stability is less than that of true species, yielding, though rarely, to degenerating or other modifying conditions.

[10] As the proof of this article passes through my hands, one of the above terms—"phenotype"—is already a matter of ancient history; while "genotype"—used here obviously in the sense in which it has been used by Jennings—has gone down, so far as this meaning is concerned, before the onslaughts of a whole series of terms, of which "biotype" is perhaps the momentary victor. With malice aforethought, the writer allows the terminology to stand as written, for the reason that, however little others may concede the point, he believes it better to retard the fixing of terms—better to keep our concepts in these fields as fluid as possible, rather than to do the reverse. Facts—on the zoological side at least—are too few; new data will certainly damage prematurely fixed definitions. Some such facts—due to the study of other rotifers and protozoa—are already known to the writer which harmonize even less than the facts of the present paper with the rigid conceptions which some set forth with show of finality.

It is worth noting—though this is in part but restating the last thought in different language—that the variations here described differ from the majority of those recently recorded for minor invertebrates;[11] for example, the modifications in *Daphnia*, *Bosmina*, and *Asplanchna*, so carefully observed by Wesenberg-Lund. These latter variations are in the main variations in external form only, and seemed to be pure reactions to external conditions, taking place, for example, when the surrounding medium has reached a certain temperature, and again lapsing very soon after the temperature has dropped. Such variations fall naturally under the rubrics of seasonal polymorphism, temporal variation, or cyclomorphosis. The variations which we have studied in *Asplanchna* refuse to be thus classified.

It is true that the stability of these variants is markedly different, being greatest for the humped type and least for the minor saccate form, but a stability that tends strongly to resist external influences is none the less obvious for each. And this seems to the writer to render it highly probable that each of these variations is of germinal origin. If this is the case it is the more striking that this germinal variation is itself a variable and elastic quantity, originally initiated by nutritive causes.

In one sense only may it be said that these mutational variations do occur in a rhythmic or cyclical fashion, in that, namely, each form may produce a fertilized or resting egg that tends to return to the common starting point. The variations are therefore obviously not transmitted through the resting egg as they are through parthenogenetic ova. It is, however, by no means certain that there is complete community of kind in all the young hatched from resting eggs. Such observations as have already been made seem to show that the progeny of the resting eggs of this species are by no means uniform,

[11] Some of the variations recorded in the recent work of Woltereck approach more closely to those here recorded for *Asplanchna*.

physiologically or morphologically. Some stocks seem larger than others from the start, and apparently gave rise more readily to the second and third types. It will require much careful experiment to ascertain the cause of these diversities, and whether a tendency toward the transmission of variations actually lies in the resting egg.

If such proves to be the case, light will be immediately thrown upon the farther problem, namely, whether the saltations here described are intimately related to a true species-making process. All in all, it seems that they probably are thus related, especially as the forms produced parallel so closely other types of the genus which are now universally regarded as definite and circumscribed species.

But are these other types of the genus definite and circumscribed species, or are they (some of them at least) but semi-independent types, occasionally brought into existence by unusual nutritive conditions and then maintaining for a time only their partial or complete autonomy? Unfortunately these remaining forms of the genus are not accessible in the writer's vicinity. But they would seem well worthy of careful study, both observational and experimental, where they may be found, and it seems to the writer that such study, sufficiently prolonged, will bring to light a species-making process in rotifers which is somewhat different from any as yet demonstrated in the animal kingdom.

It is just possible that these saltational phenomena may be purely local, or at least greatly exaggerated in the genus *Asplanchna*. The food reactions of this genus are undoubtedly extreme, and the development of their parthenogenetic ova in close proximity to this spasmodic and very variable nutritive supply may possibly make this genus exceptional. But no fundamental organic phenomenon is wholly isolated and unlike the phenomena of other species. If nutrition can modify the germ cells in the genus *Asplanchna* and thus bring into existence new types, nutrition surely must be a factor on a wider scale.